Stabilization/destabilization of cell membranes by multivalent ions: Implications for membrane fusion and division

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We propose a mechanism for the stabilization/destabilization of cell membranes by multivalent ions with an emphasis on its implications for the division and fusion of cells. We find that multivalent cations preferentially adsorbed onto a membrane *dramatically* changes the membrane stability. They not only reduce the surface charge density of the membrane but also induce a repulsive barrier to pore growth. While both of these effects lead to enhanced membrane stability against vesiculation and pore growth, the repulsive barrier arises from correlated fluctuations of the adsorbed cations and favors closure of a pore. Finally, the addition of a small amount of multivalent anions can reverse the membrane stabilization, providing an effective way to regulate membrane stability.

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Lipid bilayer membranes are resistant to rupture, primarily serving as a barrier to the leakage of the cell's contents, while also being dynamic structures that undergo various topological transitions. The capability of living cells to regulate the stability of their bounding membranes is crucial to their maintenance and reproduction [1]. Membrane stability against rupture changes most dramatically during cell division and fusion. The precise mechanism for achieving this complex task in living cells is complicated by various membrane-associated/bounded proteins [1] and is not yet clear. Numerous studies, however, suggest that membrane stability is influenced by several factors such as the ionic strength, external fields, and thermal fluctuations [2–12]. For example, red blood cells can be converted into vesicles by osmotic lysis in a solution of low ionic strength lacking multivalent cations [1,4–6]. The presence of divalent cations, however, prevents this vesiculation [4–6]. In fact, a number of experiments [4–6] have unambiguously demonstrated that the stability of red cell membranes against vesiculation can be greatly enhanced by multivalent cations. Despite this, a consistent theoretical description of this phenomenon has so far been lacking.

The strong valency dependency of membrane stability [4–6] motivated this work. Not only can osmotic lysis lead to vesiculation, but it can also create large pores in the cell membranes that subsequently contract to a size that is controlled by the ionic strength. Pore closure can be stimulated by cations, and remarkably the rate of pore closure *strongly* depends on the valency of cations [5]; Ca^{2+} is roughly 60 times as potent on a molar basis as Na^{+}. The potency of divalent cations, which essentially prevents vesiculation, was first demonstrated experimentally three decades ago [4], but its has yet to be examined theoretically. Here we propose a theoretical mechanism to explain this phenomenon. We find that multivalent counterions adsorbed onto charged membranes *dramatically* enhance the membrane stability through two effects. First, they can significantly reduce the strength of the electrostatic repulsion between backbone charges on the membrane, which enhances the membrane stability. Second, they induce a repulsive barrier to pore growth. The repulsive barrier originates from the correlated fluctuations of adsorbed counterions and favors closure of a pore, further stabilizing the membranes against vesiculation and pore growth. Upon adding a small content of multivalent anions, the adsorbed cations are released into solution, thus reversing the membrane stabilization. Adsorption/desorption of multivalent cations provide an effective way to regulate the membrane stability.

The model we consider here is a thin flat membrane [13] in the xy-plane, in the presence of a monovalent (1:1) salt such as NaCl, and in the presence or absence of Z_+-valent ($Z_+ : 1$) salts, such as $CaCl_2$. Each side of the membrane is assumed to be negatively charged with constant charge density $-e\sigma_0$ and attracts ions of the opposite charge, as schematically shown in Fig. 1(a). For simplicity, we consider the case of a single circular pore of radius R, already formed in the membrane by, for example, osmotic stress. The stability of the membrane against rupture can be quantified in terms of a line tension, i.e., the energetic penalty for creating a pore per unit length. The electrostatic repulsion between charges on the membrane favors pore formation [9], but the hydrophobic effect tends to close the pore. If mobile ions are treated as screening objects that simply reduce the electrostatic repulsion between charges on the membrane via Debye screening, then the electrostatic contribution to the line tension γ_{DH} can be estimated using Debye-Hückel (DH) theory [9]: $\gamma_{DH} \sim -e^2\sigma_0^2\kappa^{-1}\epsilon^{-1}R$, if $R < \kappa^{-1}$ and $\gamma_{DH} \sim -e^2\sigma_0^2\kappa^{-2}\epsilon^{-1}$, if $R > \kappa^{-1}$, where κ^{-1} is the screening length and ϵ is the dielectric constant of the solvent. Obviously, the electrostatic repulsion favors creation and expansion of a pore, i.e., $\gamma_{DH} < 0$.

Charged membranes are, however, capable of adsorbing counterions of the opposite charge (See Fig. 1(a)) [14,15], reducing the surface charge density of the membrane [14]. The magnitude of the reduced, renormalized charge density can be estimated by equating the chemical potentials of the "free" and "condensed" coun-

terions, i.e., those adsorbed onto the membrane surface. In the following descriptions, the subscripts $i = 1$ and 2 refer to the monovalent and multivalent counterions, respectively. If σ_i is the number density of condensed counterions, then the effective (renormalized) surface charge density on the membrane is $-e\sigma^* = -e(\sigma_0 - \sigma_1 - Z_+\sigma_2)$. The chemical potential of free counterions is mainly associated with the configurational entropy of mixing: $\mu_i^{free} \sim k_BT \ln\left(n_i a_i^3\right)$, where n_i and a_i are the concentration and size of counterions, respectively. On the other hand, the chemical potential of the condensed counterions arises from electrostatic interactions and the entropic penalty for condensation; $\mu_i^{cond} \sim -k_BT(Z_+\ell_B\sigma^*\sqrt{S}) + \ln\left(\sigma_i a_i^2\right)$ if $\kappa^{-1} > \sqrt{S}$,where S is the area of the membrane, and $\mu_i^{cond} \sim -k_BT(Z_+\ell_B\sigma^*\kappa^{-1}) + \ln\left(\sigma_i a_i^2\right)$ otherwise. The equilibrium values of σ_i can then be obtained by requiring $\mu_i^{free} = \mu_i^{cond}$.

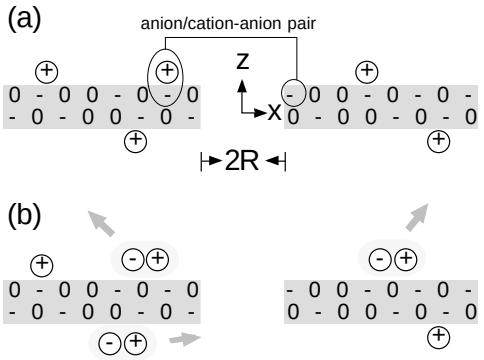

FIG. 1. (a) Schematic view of a charged membrane with a pore of a radius R. Charged and neutral lipids are denoted by $-$ and 0, respectively, while the adsorbed multivalent cations are denoted by encircled $+$'s. A pair of attracting lipid and lipid/cation is also shown that stabilizes the membrane against pore growth. (b) Illustration of multivalent anions (encircled $-$'s) forming pairs with the adsorbed multivalent cations, then leaving into solution. Depletion of the adsorbed layer destabilizes the membrane.

The condensed counterions do not simply renormalize the membrane charge density but also give rise to charge fluctuations in the plane of the membrane surface that tend to be correlated with each other [16,18]. A typical attracting pair of a lipid and lipid/cation is illustrated in Fig. 1. Creation of a pore makes charges at the edge less efficiently correlated and is discouraged by the charge correlation effects. Computation of the charge correlation contribution to the pore free energy is highly involved, partly because the charge fluctuation interactions are not pairwise additive [16,17]. This is complicated by yet another factor: the specific geometry of the membrane with a pore. The electrostatic effects at the meanfield level, suppressing both adsorption and charge fluctuations, has only recently been addressed [9]. To study the effects of charge fluctuations on the membrane stability, we take the continuum limit and incorporate the in-plane charge fluctuations at the Gaussian level as in previous cases [16,18]. The resulting charge fluctuation contribution to the pore free energy, i.e., the change in the charge fluctuation free energy by creating a pore, is formally given by

$$\frac{\Delta\mathcal{F}_{pore}}{k_BT} = \tfrac{1}{2}\ln\det\left[1 + (Q - \mathcal{Q})\mathcal{Q}^{-1}\right]. \tag{1}$$

Here, the matrix Q is defined by the matrix elements

$$Q_{\mathbf{x}_\perp\mathbf{x}'_\perp} = 1 + \ell_B\sigma_{cc}\zeta_{\mathbf{x}_\perp\mathbf{x}'_\perp}\frac{\mathrm{e}^{-\kappa|\mathbf{x}_\perp - \mathbf{x}'_\perp|}}{|\mathbf{x}_\perp - \mathbf{x}'_\perp|}, \tag{2}$$

where $\mathbf{x}_\perp = (x, y)$, $\ell_B = e^2/\epsilon k_BT$ is the Bjerrum length, $\sigma_{cc} \equiv \sigma_1 + Z_+\sigma_2$, $\kappa^2 = 8\pi\ell_B I$, I is the ionic strength of the solution, and $\mathcal{Q} \equiv \lim_{R\to 0} Q$. Finally $\zeta_{\mathbf{x}_\perp\mathbf{x}'_\perp} = 1$, if $\mathbf{x}_\perp$ and $\mathbf{x}'_\perp$ are on the membrane and is 0 otherwise. In the case of $S > \kappa^{-1}$, as is the case for red cell experiments [4,5], $\mathcal{F}_{pore}$ in Eq. (1) can be calculated without making further approximations. This follows from the fact that $\delta \equiv (Q - \mathcal{Q})\mathcal{Q}^{-1} \sim S^{-1}$ and $\mathcal{F}_{pore}$ can then be expanded in powers of δ. In the case $S > \kappa^{-1}$, we can take the limit $S \to \infty$ without introducing any appreciable error. In this limit, only the leading term survives in the expansion.

To calculate the free energy in Eq. (1), it proves useful to Fourier transform it from $\mathbf{x}_\perp$ to $\mathbf{k}_\perp$. We find

$$\begin{aligned}\frac{\Delta\mathcal{F}_{pore}}{k_BT} \simeq \frac{1}{2\lambda_{cc}}\left[\int\int_{R=0} - \int\int_{R>0}\right] rdrr'dr' \\ \times \int_0^{2\pi} d\theta \int_0^\infty \frac{k_\perp dk_\perp}{1 + \lambda_{cc}\sqrt{k_\perp^2 + \kappa^2}} \cdot \frac{\mathrm{e}^{-\kappa\sqrt{r^2 + r'^2 - 2rr'\cos\theta}}}{\sqrt{r^2 + r'^2 - 2rr'\cos\theta}} \\ \times J_0(k_\perp\sqrt{r^2 + r'^2 - 2rr'\cos\theta})\end{aligned} \tag{3}$$

where $\lambda_{cc} \equiv 1/2\pi\ell_B\sigma_{cc}$ and $J_0(x)$ is the zeroth-order Bessel function of the first kind. In the case of $\kappa R \gg 1$, the charge-fluctuation line tension, i.e., $\Delta\gamma = \Delta\mathcal{F}_{pore}/2\pi R$, shows two distinct scaling behaviors: $\Delta\gamma \sim \lambda_{cc}^{-1}\ln(1/\lambda_{cc}\kappa)$ for small $\lambda_{cc}\kappa$ and $\Delta\gamma \sim \lambda_{cc}^{-2}\kappa^{-1}$ for large $\lambda_{cc}\kappa$.

The charge fluctuation ($\Delta\gamma > 0$) and hydrophobic contributions ($\gamma_0 > 0$) favor closure of the pore, while the DH ($\gamma_{DH} < 0$) or renormalized DH term ($\gamma^*_{DH} < 0$) tends to expand the pore. To study the membrane stability, we have solved for σ^* and $\gamma_{total} \equiv \gamma_0 + \gamma^*$ (or γ^*_{DH}) + $\Delta\gamma$ simultaneously. We have chosen $a_1 = a_2 = 2$Å, $T = 300$, and $\sigma_0 = 0.2\mathrm{nm}^{-2}$, which is in the physiological range. To suppress the R-dependency, we have assumed that $R > \kappa^{-1}$. Fig. 2 shows γ_{total} in units of $\gamma_0 = 10^{-11}$J/m [8], as a function of the monovalent counterion concentration n_1. In the absence of multivalent counterions ($Z = 1$), γ_{total} is negative when n_1 is in the range $0 \le n_1 \le 1$mM. This implies that the membrane is unstable to pore growth as long as n_1 is in this range. The presence of 0.1mM of multivalent counterions ($Z = 2$ and 3), however, dramatically enhances the membrane stability. In this case, γ_{total} is positive for the whole range of

n_1 and is in the range $0.6\gamma_0 \leq \gamma_{total} \leq 0.7\gamma_0$. In order to enhance the membrane stability up to this level by monovalent counterions, about 5mM concentration would be needed. This is approximately 50 times higher than that of the divalent counterion concentration. This estimate is remarkably consistent with the experimental finding that Ca^{2+} is roughly 60 times more effective on a molar basis than Na^+ in stimulating pore closure [5].

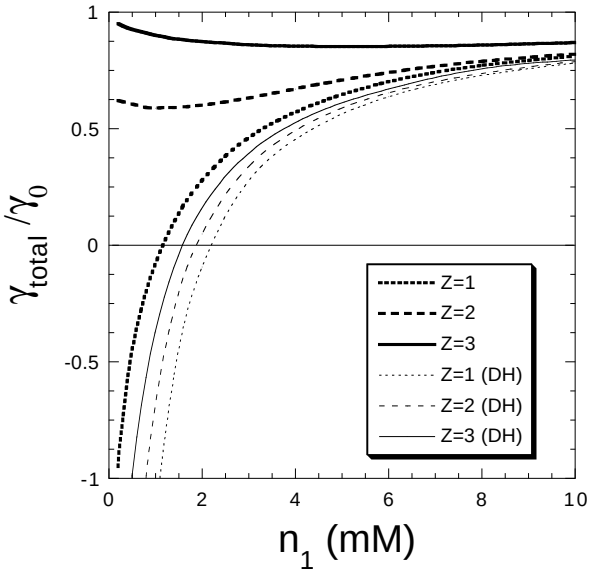

FIG. 2. Total line tension, in units of the hydrophobic contribution $\gamma_0 = 10^{-11}$J/m, as a function of the monovalent salt concentration n_1. We have chosen $T = 300$ and $\sigma_0 = 0.2\text{nm}^{-2}$. In the absence of multivalent cations ($Z = 1$), there exists a finite range of the monovalent salt concentration where the membrane is unstable ($\gamma_{total} < 0$) to pore formation. The presence of as small a concentration as 0.1mM of multivalent cations ($Z = 2, 3$) stabilizes the membrane against pore growth for the whole range of monovalent salt concentration. The distinction between the monovalent and multivalent cases is, however, minor in the DH approach, and 0.1 mM of multivalent cations only *slightly* enhances the membrane stability.

Our results are striking; the presence of multivalent counterions is more crucial to the membrane stability than that of the monovalent salt, though the ionic strength is mainly determined by the latter. This can, however, be understood in the context of "counterion condensation". The long-ranged electrostatic interactions allow the membrane to adsorb multivalent counterions *preferentially* even when $n_2 \ll n_1$. These condensed multivalent counterions not only reduce the repulsion between backbone charges on the membrane, but they also enhance the strength of charge fluctuations—both of these effects are more efficient with multivalent cations than with monovalent ones. When combined, these two effects lead to significantly enhanced membrane stability against pore growth and vesiculation. Also note that trivalent counterions are even more efficient in enhancing the membrane stability than the divalent counterions. As shown in the figure, the dramatic distinction between the monovalent and multivalent cases is missing in the DH theory. The enhanced membrane stability by multivalent counterions seen in the experiments [4,5] can be explained *only* when both the preferential adsorption of multivalent counterions and the effects of charge correlations are properly taken into account.

Whether a pore grows or closes also depends on the height of the barrier as a function of the pore size R. In Fig. 3, we have plotted the pore free energy as a function of R, in units of k_BT. We have chosen $T = 300K$ and $\sigma_0 = 0.2\text{nm}^{-2}$. The barrier height is finite in the presence of monovalent ions only ($Z = 1$). In contrast, the pore free energy in the presence of 0.1mM of multivalent counterions ($Z = 2, 3$) grows *indefinitely* with R. This implies that formation of a large pore is energetically greatly *dis*favored, in the presence of multivalent counterions. The results are indeed consistent with the experimental observation that the presence of 0.1mM of $MgSO_4$ stabilized the red blood cell ghosts against vesiculation [4]; a pore originally created by osmotic stress will grow into a large one or shut down, depending on the buffer quality and the strength of the restoring force provided by the spectrin network. Note that the osmotic stress will eventually be removed. When $Z = 1$, the pore can grow into a large one, once the barrier is overcome by osmotic stress. This will lead to vesiculation if the restoring force is outweighed by the repulsion between the charged groups on the red cell membranes. In contrast, there is a subsequent barrier to pore growth in the case $Z = 2, 3$. This prevents the ghosts from breaking into vesicles. In contrast, the DH approach mistakenly implies that the barrier height is roughly *in*sensitive to the valency of counterions. Thus our results in Fig. 3 further support the importance of the counterion valency and charge correlations to the membrane stability, consistent with experiments [4,5].

The fact that multivalent cations can be preferentially adsorbed onto a charged surface implies that the layer of the adsorbed cations can be depleted by multivalent anions. Imagine an anion of valency Z_- making a pair with a cation in the condensed layer and leaving into the solution, as illustrated in Fig. 1(b). Whether this is feasible can be tested by calculating the change in the chemical potential: $\Delta\mu \sim -Z_+Z_-\ell_B/\left(a_{2+}+a_{2-}\right)+Z_+\ell_B\sigma^*\kappa^{-1}+\ln\left(n_{2-}a_{2-}^3/n_{2+}a_{2+}^3\right)$, where the subscript 2 refers to multivalent ions and the subscripts + and − refer to the cations and anions, respectively. When the valency of anions is sufficiently high, this change can be negative. This implies that a certain fraction of the cations in the layer make pairs with multivalent anions and will return to the solution to maintain "chemical equilibrium". Note that a minimum concentration of multivalent anions is required for this process to occur. This can be readily seen by taking the limit $n_{2-} \to 0$ and noting that, in this limit, the entropic penalty for pairing is too large. Since only a very small concentration of multivalent cations

is needed to enhance the membrane stability, the presence of an equally small content of multivalent anions suffices to deplete the condensed layer, effectively reducing the membrane stability. Cells contain multivalent cations (e.g. Ca^{2+} and Mg^{2+}) as well as multivalent anions (e.g., PO_4^{3-} and anionic proteins) and thus could regulate their stability in this way. This may provide a new insight into the biological phenomena of "breakdown" and "reassembly" of a nuclear envelope during cell division. During cell division, a nuclear envelope disintegrates into vesicles, which eventually reassemble into daughter cells [1]. This is suggestive of the cyclic change in the membrane stability: stable → unstable → stable. The stabilization/destabilization of membranes by multivalent ions could be relevant to this flow of membrane stability in a dividing cell. This is, however, complicated by various membrane-bounded proteins [1] and further consideration is certainly warranted.

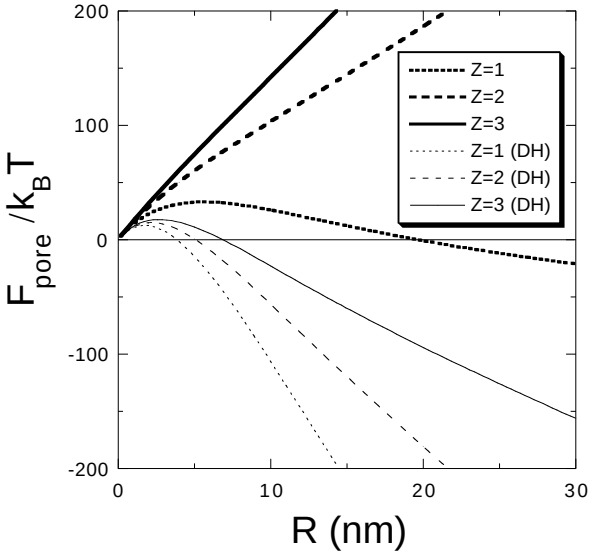

FIG. 3. Pore free energy as a function of the pore size R. We have chosen the same parameters as were used to generate Fig. 2. The pore free energy is estimated in units of k_BT. For the monovalent salt case ($Z = 1$), the pore free energy has a finite barrier. In the presence of 0.1mM of multivalent counterions ($Z = 2, 3$), the pore free energy grows *indefinitely* with R. In the DH approach, the barrier height is roughly *in*sensitive to the counterion valency Z.

To summarize, we have presented a working mechanism for the stabilization/destabilization of membranes by multivalent ions. Multivalent cations preferentially adsorbed onto a charged membrane not only reduce the surface charge density of the membrane but also induce a repulsive barrier to pore growth that favors the closure of a pore. The main advantage of the membrane stabilization by multivalent cations lies in that this can be easily reversed; the addition of a small concentration of multivalent anions can reverse the membrane stabilization. Our results are also consistent with the experimental findings that membrane adhesion by multivalent cations does not necessarily lead to membrane fusion and that membrane fusion (in the absence of fusion peptides) should be followed by lateral phase separation of the lipid molecules into two distinct phases [15]: anionic lipid-poor phases and anionic lipid-rich phases "coated" with multivalent cations. It is the *un*coated phases that undergo a topological change and eventually fuse into each other, consistent with our picture of multivalent cations as efficient agents for stabilizing membranes against rupture.

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